Complex effects of non-host diversity on the removal of free-living infective stages of parasites

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Introduction

Transmission pathways of parasites and pathogens can be altered by surrounding ecological communities, ultimately affecting disease dynamics. Recent research into such effects of communities on parasite transmission has identified several mechanisms of how surrounding ecological communities can affect disease risk in focal hosts. These mechanisms relate to a phenomenon called the *dilution effect*, which is the theoretical concept that increased biodiversity results in a reduction in disease risk (Keesing *et al.* 2006; Ostfeld & Keesing 2012). While there are several mechanisms that can lead to a biodiversity-mediated alteration of disease risk (Keesing *et al.* 2006), one of the mechanisms receiving most attention has been *encounter reduction,* which is when surrounding communities cause a reduction in encounters between susceptible and infectious hosts or infective stages. The most prominent examples of encounter reduction come from vector-borne diseases with frequency–dependent transmission, such as Lyme disease, in which hosts of lower competence can act as decoys for vectors and pathogens. Thereby, the pathogen pool becomes diluted, leading to reduced prevalence in focal hosts (Ostfeld & Keesing 2000, 2012). A second line of research expanded on the encounter reduction theory by assessing how changes in the diversity of hosts with different competence affect non-vector borne diseases with density-dependent transmission. Most of this work has been conducted with free-living cercarial stages of trematodes that infect tadpoles and has indicated that less competent hosts can act as decoys for infective stages, thereby lowering infection levels in the main competent host (Johnson *et al.* 2008a, 2013). Finally, a third line of research has been focussing on how non-hosts

(i.e. organisms which do not serve as competent host or less competent decoys and thus do not become infected) can interfere with the transmission of free-living infective stages (Thieltges *et al.* 2008; Johnson & Thieltges 2010). This interference can, for example, occur when non-hosts prey on free-living infective stages or act as a physical obstruction. The removal of parasites from the pool of infective stages subsequently leads to reduced infection levels in focal hosts (Johnson & Thieltges 2010; Johnson *et al.* 2010; Goedknegt *et al.* 2015). This form of transmission interference is probably widespread and does not only affect free-living infective stages of macroparasites but also inhibits the transmission of microparasites such as viruses (Welsh *et al.* 2020).

Although there is no doubt that many organisms can affect disease transmission and dynamics via the mechanisms discussed above, whether a reduction in disease risk with an increase in diversity (dilution effect) is a universal phenomenon or whether diversity effects are instead idiosyncratic is still under debate (Randolph & Dobson 2012; Lafferty & Wood 2013; Ostfeld & Keesing 2013; Salkeld *et al.* 2013; Wood & Lafferty 2013; Civitello *et al.* 2015; Johnson *et al.* 2015). In the case of vector-borne diseases such as Lyme disease, dilution effects have been reported from several disease systems, including aquatic and terrestrial systems (Ostfeld & Keesing 2012). However, mechanisms linking biodiversity and disease risk for focal hosts may actually be more complex and may, result not only in a reduction but also in an amplification of disease risk depending on the specific circumstances such as habitat changes, host densities and spatial scales (Wood & Lafferty 2013). Therefore, experimental manipulations rather than field observations have been better suited to disentangle the effects of host competency on disease risk, especially in the case of parasites with density-dependent transmission (Johnson *et al.* 2013, 2015). In addition, experiments also allow for the separation of true diversity effects from density effects as simply increasing the density of a host species may have the same effect as increasing diversity. For example,

experiments with the fungal pathogen *Batrachochytrium dendrobatidis* showed a decrease in infection levels in the focal amphibian host in communities with increased richness of less competent hosts which was independent of host density (Searle *et al.* 2011). Similarly, experiments with the trematode *Ribeiroia ondatrae* infecting amphibians showed a decline in tadpole infection levels in the presence (and at the same total host density levels) of another amphibian species with lower host competence (Johnson *et al.* 2008a).

While experimental studies on the effects of community composition of less competent decoy hosts on disease risk are increasing, experimental studies on the third type of encounter reduction, the removal of infective stages by non-hosts, are very limited. In one experimental study involving trematode cercariae infecting tadpoles, diversity and density of non-host odonate larvae affected the transmission of cercarial stages (Rohr *et al.* 2015). However, reduced infection levels in focal hosts were probably not only the result of parasite removal but also of predation by larvae on tadpoles and non-consumptive predator effects in form of fear-induced behavioural changes of the hosts (Rohr *et al.* 2015). In general, studies have shown that a multitude of non-host species can remove infective stages of a large range of parasite groups (Thieltges *et al.* 2008) and also that this removal can be non-host density-dependent (Thieltges *et al.* 2009; Rohr *et al.* 2015). However, whether the addition of other non-hosts to experimental communities' results in true diversity-mediated and not just density effects has not little studied. In part, this is probably related to the methodological difficulties in conducting meaningful comparisons of non-hosts of very different morphologies, sizes and parasite removal mechanisms (Johnson & Thieltges 2010). This scarcity of studies hampers our understanding of the mechanisms underlying the general relationship between biodiversity and disease (Johnson *et al.* 2015).

In this study, we used an experimental approach from general community ecology, the response surface design, to overcome methodological issues in disentangling diversity and density effects of communities of different non-host taxa organisms with different parasite removal mechanisms. Typically, response surface design experiments incorporate two different competitive species at various densities and thus combines additive and substitutive experimental designs (Inouye 2001; Fig. 1). This design allows for the statistical testing of inter- and intraspecific interactions and is therefore suitable to disentangle the effects of species diversity from density effects. For our laboratory experiments, we used cercariae of a common marine trematode species (*Himasthla elongata*), a parasite with a complex life cycle. This species uses periwinkles (*Littorina littorea*) as first intermediate hosts, from which cercariae are released into the water column and subsequently infect (as metacercarial cysts) a second intermediate bivalve host, such as the blue mussel *Mytilus edulis*. Here, via the predation of the bivalve by a definitive bird host, the parasite is able to develop into its adult stage and sexually reproduces inside the bird, after which eggs are released with the host's faeces (Werding 1969). Infection intensity in the second intermediate host is dose-dependent, i.e. the number of metacercarial cysts in a host is positively correlated with the number of infective cercarial stages the host has been exposed to (Welsh *et al.* 2017). Metacercarial infections in the second intermediate host result in reduced fitness in form of a decrease in condition, filtration rates and growth with negative effects generally being considered to be density-dependent (Thieltges 2006; Stier *et al.* 2015). Hence, in this marine trematode system, any alterations in the number of infective stages (cercariae) by non-hosts will affect infection levels (metacercariae) and associated disease risk for the second intermediate bivalve host. To study the effect of non-host diversity on parasite removal we used three non-host species from widely different taxa that are common in coastal waters and have been shown to interfere with cercarial transmission via different removal mechanisms: the predatory shore crab *Carcinus maenas*; the filter feeding Pacific oyster

Crassostrea gigas and a physical trap in form of the seaweed *Sargassum muticum* (Thieltges *et al.* 2008; Welsh *et al.* 2014). Instead of using the infection levels in second intermediate hosts to identify non-host diversity effects related to parasite removal, we determined the number of remaining free-living parasite stages after removal of the non-host communities. Therefore, the results were not confounded by predation of second intermediate hosts by non-hosts or non-consumptive effects such as behavioural changes of parasites and second intermediate hosts in presence of non-hosts. We hypothesised that the addition of a second non-host species will result in additive parasite removal and thus a reduction in disease risk.

Materials and methods

Experimental organisms

To obtain sources of cercariae, we collected periwinkles (*Littorina littorea*) from the intertidal area in the vicinity of the NIOZ Royal Netherlands Institute for Sea Research on Texel (Wadden Sea, The Netherlands). Snails infected with *Himasthla elongata* were identified by shedding trials (release of cercariae under light at 27°C), kept in aerated flow-through aquaria and fed with sea lettuce (*Ulva lactuca*). For the experiments, we obtained cercariae from infected snails by incubating approximately 30 snails under light at 27°C in 3 L of seawater for 3 hours. The required amount of cercariae was then pipetted and administered to the experimental units within one hour (i.e. cercariae were not older than 4 h at the start of the experiment).

Three non-host species which all coexist in the same habitat study areas (e.g. mixed mussel and oyster beds, dykes) were used: Pacific oysters (*Crassostrea gigas*) which are sessile filter feeders, shore crabs (*Carcinus maenas*) which are motile active predators and seaweed (*Sargassum muticum*), which forms a physical obstruction for cercariae. The sizes of non-hosts reflected

common size ranges observed in the field: Pacific oysters: 10.51 ±1.2 cm widest diameter, shore crabs: 3.1 ± 0.3 cm carapace width, and seaweed: branches of individual plants. All three species were previously identified as interfering with *H. elongata* transmission (Thieltges *et al.* 2008; Welsh *et al.* 2014) and were collected from the intertidal and shallow subtidal area in the vicinity of the NIOZ Royal Netherlands Institute for Sea Research on Texel (Wadden Sea, The Netherlands). Immediately after collection, any epibionts were carefully removed and all organisms were kept in aerated flow through aquaria in the same climate chamber at 18°C. Crabs were fed on a diet of mussels while oysters were fed algal bivalve feed (*Isochrysis galbana*, Instant Algae by Reed Mariculture Inc. USA; 4.1 billion cells ml^{-1}; administered as 4 drops of algal feed per oyster, as recommended by Reed Mariculture).

Experimental design

To test for the effects of non-host diversity on the removal of cercariae we used a two-factorial response surface design, with two different non-host species and four density levels (Fig. 1). This design combined both additive (varying non-host diversity but also density at the same time) and substitutive (varying diversity but keeping density constant) designs and allowed us to separate diversity from density effects as well as identify potential interactive effects between both factors (Inouye 2001; Fig. S1). Density levels of the three non-host species reflected densities that can be locally observed in the field (pers. obs.) and were as follows: oysters (0, 1, 2, 6 ind.), crabs (0, 1, 2, 3 ind.), and seaweed (0, 5, 15, 30 g fresh weight after gently drying with a paper towel). The treatment with zero densities of both non-host species served as a control for potential background losses of cercariae.

All experiments were carried out in separate runs in a temperature- and light-controlled room (18.0°C ± 0.2°C; 10:14 hour light/dark cycle). Each experiment tested two different non-host species at four density levels and each treatment was replicated four times (i.e. 64 replicate units in total per run; Fig. 1). Each of the replicate units consisted of a 2 L aquarium with 1500 ml of filtered seawater. To allow for acclimation, all test organisms were starved and kept in the experimental containers for 24 hours prior to the experiment starting. At the start of the experiment, 100 cercariae were added to each experimental unit and the aquaria were left undisturbed for the following three hours. After three hours, the experiment was terminated by quickly removing all non-hosts with forceps. The water from each experimental unit was filtered through a 25µm sieve to retain any remaining cercariae. The units were then flushed with filtered seawater and sieved a further two times to reduce chances of cercarial adhesion to the walls of the units. Subsequently, the cercariae were washed from the sieve and fixed using 10 ml of 96% ethanol and stained using Rose Bengal. After a minimum of 24 hours to allow sufficient staining, all cercariae were counted in Petri dishes under a stereo microscope.

Data visualisation and statistical analysis

To visualise the results, we plotted the mean absolute numbers of cercariae remaining per treatment combination by placing the different density levels of one of the two non-host species on the x-axis and separating the different densities of the second non-host species into four different line series. Although combining data points with lines in a categorical design is usually not appropriate, this graphical depiction allowed for an easy visualisation of potential interactions between the two main factors. Parallel lines would suggest additive effects of a second non-host species while crossing or diverging lines would indicate interaction effects of the two non-host species on cercarial removal.

The effects of non-host species on cercarial removal was investigated using binomial Generalized Linear Models (GLMs) with a log-link. We assumed a *linear pure death process* (i.e. each cercarial removal by non-hosts is an independent event) so that the number of cercarial stages remaining at the end of the experiment is binomially distributed, with a probability of cercariae surviving until the end being equal to $p=e^{-\theta t}$ where θ is the rate at which cercariae are removed per unit of experimental time, and hence t=1. This cercarial removal rate was assumed to be a function of non-host diversity, density and the interaction between both, thus:

$$\theta=\mu+\alpha_i+\beta_j+\gamma_{ij}$$

where α_i represents the effect of the first non-host at the i^{th} density, β of the second non-host at the j^{th} density, and γ_{ij} their interaction.

We then fitted a series of GLMs from the most complex to the least complex model (for an illustration of the model selection procedure see Fig. S1). In the most complex model, all explanatory variables were included (including the interaction) while the simplest model only contained the intercept (null model). Using analysis of deviance, we identified the best fitting model by testing for significant differences between models of decreasing complexity. To illustrate the procedure, the most complex model was tested against the next less complex model (including the effects of both species but not their interaction). The difference in deviance (delta deviance; Δ Dev) between the two models was then divided by the dispersion factor (ϕ; most complex model residual deviance divided by degrees of freedom) and compared to the delta degree of freedom χ^2 at 0.05 to identify statistical significance. A significant difference between two models indicated a better fit of the more complex model. Using the model coefficients and unique estimates of intercepts for each of the factors included in the best fitting model, we calculated cercarial removal rates and

parasite survival (%). All analyses were carried out using R (R Core Development Team 2019) version 3.0.2 in R Studio (R Studio 2018).

Results

General patterns

For all three combinations of non-host species, the best-fitting models were the most complex ones which included the interaction between both non-host species. Thus, the effect of a specific non-host species on cercarial removal depended on the density of the other non-host species (Table 1; Fig. 2). Data visualisation by plotting the mean absolute numbers of cercariae remaining at different non-host density levels for one species against that of the second species (Fig. 2) revealed diverging, converging and crossing of the lines, thus denoting the presence of interactions between the first and second non-host species. Therefore, depending on the non-host species combination and the density levels, the presence of a second non-host species resulted in a neutralisation, amplification or reduction of the parasite removal effects exerted by the first non-host species. When comparing the same non-host species across all three experiments and at the same density, we observed slight differences in cercarial removal rates, however, the general removal patterns were similar among the different experiments (Fig. S2).

Crabs and seaweed experiment

In the experiment using crabs and seaweed, the mean number of infective stages remaining at the end of the experiment decreased with increasing crab and seaweed density (Fig. 2A). At low densities of crabs (0-1 ind.), the addition of seaweed to the experimental units had an additive effect (suggested by the roughly parallel lines), while at higher crab densities (2-3 ind.) survival of cercariae strongly decreased with increasing seaweed densities (diverging lines; Fig. 2A). The

survival of cercariae was lowest (28 %) and thus the total removal rate by the non-hosts highest (1.26; calculated from the best-fitting model; Table S1) in the treatment with the highest crab and seaweed density levels (Fig. 2A). In absence of seaweed, increasing crab densities lead to a decrease in the number of cercariae remaining, however at the highest crab density level (3 crabs), cercarial survival increased again, leading to a trough-shaped curve in the numbers of cercariae remaining (Fig. 2A; Table S1). In the absence of crabs, cercarial survival decreased with increasing seaweed density (0-15 g), however, at the highest seaweed density level (30 g) cercarial survival was relatively similar to the one observed at the second highest density level (15 g; 73% and 76%, respectively; calculated from the best-fitting model; Table S1).

Seaweed and oysters experiment

In the experiment investigating the effects of seaweed and oysters, in absence of oysters the mean number of surviving infective stages remaining at the end of the experiment decreased with increasing seaweed density (Fig. 2B). Likewise, when seaweed was absent, the number of remaining cercariae decreased with increasing oyster densities (Fig. 2B). However, when oysters and seaweed were combined, total cercarial removal rates were lower than in oyster only treatments. In addition, the difference in cercarial removal between seaweed only and mixed treatments decreased with increasing seaweed densities (converging lines; Fig. 2B). At the highest seaweed density, the three treatments with oysters showed similar cercarial survival of approximately 60% (calculated from the best-fitting model; Table S2) as the seaweed only treatment (Fig. 2B).

Oysters and crabs experiment

Finally, in the experiment investigating oysters and crabs we observed a crossing of the different lines (Fig. 2C). In absence of crabs, cercarial removal decreased with increasing oyster density, however, the addition of crabs to the experimental units resulted in a much lower effect of oyster density on cercarial removal (Fig. 2C). While cercarial removal decreased with increasing crab density in absence of oysters, this pattern was fully reversed at the highest oyster density (6 ind.), with cercarial removal increasing with increased crab density and thus the highest cercarial survival was observed at the highest crab density (Fig. 2C; Table S3). Alike the experiment with crabs and seaweed, cercarial survival in absence of the second non-host species decreased with crab density but then, albeit less strongly, increased again at the highest crab density (3 crabs; 63%, calculated from the best-fitting model; Table S3) compared to the survival at the intermediate crab density (2 crabs; 60%; Table S3; Fig. 2C).

Discussion

In all three experiments, total parasite removal by the experimental non-host communities was a function of the interactive effects between the two non-host species. However, adding a second non-host species to the experimental units did not universally result in an increase in total parasite removal, thus contradicting the expected reduction in disease risk with increasing non-host diversity. Instead, increasing non-host diversity by adding second species led to a neutralisation, amplification or reduction of the parasite removal effects exerted by the first non-host species, depending on the respective densities and non-host species combination. This suggests that diversity effects, in respect to parasite removal by non-hosts, exist independent of density effects but that the direction and magnitude of these effects are idiosyncratic and strongly conditional on the respective non-host combinations.

These idiosyncratic effects of non-host diversity on parasite removal probably resulted from interactions among non-host species arising in the different non-host combinations. Adding a second non-host species to experimental units is likely to affect the behaviour of one or both non-host species, with potential effects on the total parasite removal of the community. In the case of the crab-seaweed combination, it is likely that the addition of seaweed to the experimental units allowed the crabs to move through the seaweed matrix. This way they could access cercarial stages which were swirling higher up in the water column that would otherwise have been unreachable if no seaweed were present (crabs remove cercariae via their mouth parts and gills (Welsh *et al.* 2019). As higher densities of seaweed provided a denser matrix for the crabs, which themselves removed cercariae in a density-dependent fashion, removal rates in the combined treatments increased with increasing crab and seaweed densities and community removal rates were highest in the treatments with the highest crab and seaweed densities. Hence, through such interspecific interactions the addition of seaweed may have strongly amplified the parasite removal effect exerted by crabs alone. In contrast, in the oyster-seaweed combination, the matrix created by the seaweed did not result in increased parasite removal by oysters, instead with an increase in seaweed density the seaweed probably trapped more and more cercariae so that fewer cercariae made it to the bottom of the experimental units where the oysters were positioned (oysters remove cercariae by filtration; Welsh *et al.* 2019). Hence, in this case, the addition of seaweed lead to a neutralisation of the parasite removal effects exerted by oysters alone. Finally, in the case of the oyster-crab combination, the addition of crabs, a known predator of Pacific oysters (Mascaró & Seed 2000; Mascaro & Seed 2001), lead to a reduction of the parasite removal effects exerted by oysters alone, possibly because the movements of crabs throughout the experimental units disturbed the oysters inducing valve closure and thus reduced their filtration activity. The interspecific interaction between oysters and crabs likely increased with crab density. This would explain our observation that, at the highest

seaweed density, cercarial survival was highest at the highest crab density and thus quashed the pattern of highest cercarial survival at lowest crab densities when seaweed was absent. We acknowledge that further experiments will be needed to verify the suggested interactions, however, it is plausible that differential behavioural changes initiated by the addition of a second non-host species underlie the idiosyncratic diversity effects observed in our experiments.

In addition to interspecific interactions, intraspecific interactions among individuals of the same non-host species may have further modified total parasite removal rates in the experimental communities. For example, crabs showed slightly lower parasite removal rates at high densities compared to intermediate crab densities (albeit less pronounced in one of the two experiments with crabs). This may have resulted from intraspecific interactions among crabs which are known to show aggressive display and fighting behaviour in presence of conspecifics, which can lead to reduced predation rates due to interference competition (Smallegange *et al.* 2006). Similar interference interactions may have occurred in our experiments leading to a reduction in parasite removal rates at high crab densities. Such intraspecific interactions may also have an individual component as individual crabs differ in competitive strength (Sneddon *et al.* 2000) and this may explain the slight variation in removal rates at the highest crab densities between the two experiments involving crabs. Whatever the exact mechanisms, our experiments clearly indicated that non-host diversity effects resulted in different directions and magnitude in the three non-host combinations.

The different non-host diversity effects observed in the experiments translate into different disease risks for the downstream host in the life cycle. As infection levels in the second intermediate host are dose-dependent for the parasite-host system used in our experiments (Welsh *et al.* 2017), any

311 alteration of the number of infective stages will affect host infection levels and, as metacercarial
312 infections are intensity-dependent (Thieltges 2006), also alter the disease risk for the host. Hence,
313 depending on the outcome of the effects of intra- and interspecific interactions between non-host
314 species on the total parasite removal, focal hosts are likely to experience very different parasite
315 pressures and associated disease risks. This conclusion seems to contradict findings from meta-
316 analyses based on published studies on diversity effects on disease risk which found evidence for
317 the generality of dilution effects among diverse host and disease systems (Civitello *et al.* 2015;
318 Huang *et al.* 2017). However, the database underlying these analyses included a broad variety of
319 studies, many of which only studied the effect of the addition of a single less competent host species
320 on parasite transmission rather than effects of more complex communities of less competent hosts
321 or non-hosts. In addition, most of these studies were not designed to disentangle diversity from
322 density effects. Studies that tried to separate diversity from density effects are surprisingly rare and
323 paint a more complex picture. Some studies that investigated diversity effects of hosts of
324 differential competence found diversity effects for both fungal pathogens and trematodes infecting
325 amphibian hosts (Johnson *et al.* 2008b; Searle *et al.* 2011). In contrast, experiments using
326 substitutive deigns (i.e. varying diversity while keeping density constant) on cercarial predation by
327 three larval odonate species did not reveal diversity effects (Rohr *et al.* 2015) However, in a
328 mescosom experiment of the same study which included snails as sources for cercariae, the odonate
329 cercararial predators and the down-stream hosts, diversity effects were observed, albeit depending
330 on odonate density (Rohr *et al.* 2015). In this case, the effect was not only the result of parasite
331 removal but also of predation on focal hosts by some of the odonate species and non-consumptive
332 predator effect in form of fear-induced behavioural changes in hosts (Rohr *et al.* 2015). This
333 suggests that the addition of hosts to experimental units adds yet another layer of diversity-mediated

334 effects, further suggesting that diversity-disease relationships are probably highly conditional on
335 the disease system at hand.
336
337 With such a complexity of factors modifying the relationship between diversity and disease already
338 in relatively simple experimental settings, the question arises to what extent diversity effects can
339 also be observed in the field. Regarding the parasite-host system investigated in our experiments,
340 large-scale investigations of the correlates of infection levels in mussels living on mixed mussel
341 and oysters beds in our study area did not reveal evidence for dilution effects of oysters on
342 infections of mussels with the trematode species we used in our experiments (Goedknegt *et al.*
343 2019). However, this does not mean that disease-mediated effects do not occur under natural
344 conditions as field experiments in the same area have shown a decrease in infection levels in
345 mussels in the presence of oysters (Thieltges *et al.* 2009). Instead, it is more likely that the
346 complexity of species interactions with direct and indirect effects on parasite transmission hampers
347 the detection of specific diversity effects in the field. This may be further exacerbated in marine
348 ecosystems, where wide-spread parasite dispersal can occur but may also be subjected to additional
349 mediating effects, such as those caused by tides, ocean currents and other physical dynamics.
350 Studies in much more closed ecosystems such as freshwater lakes and wetlands have been more
351 successful in finding some evidence for diversity-mediated effects on parasite infections level s
352 (Lagrue & Poulin 2015; Rohr *et al.* 2015). In contrast to these findings but more similar to our
353 research, a meta-analysis of field studies on the relationship between diversity and zoonotic
354 diseases in terrestrial ecosystems only found weak and idiosyncratic diversity effects (Salkeld *et*
355 *al.* 2013). However, whether there really are differences in the relevance and strength of diversity-
356 disease relationships among the major realms still remains to be investigated. Therefore, more
357 studies from different host and disease systems, ideally combining experimental and correlative

358 field approaches, are needed to identify any potential general patterns in the direction and strength
359 of diversity effects on disease risk.
360
361 **Conclusions**
362 Our experiments revealed non-host diversity effects on parasite removal. However, these diversity
363 effects did not generally result in a reduction of disease risk, instead the direction and magnitude
364 of changes in disease risk were idiosyncratic, probably driven by intra- and interspecific
365 interactions among the different non-host species in the experimental communities. Given the
366 likelihood of a wide range of species interactions in natural communities, non-host diversity effects
367 on parasite removal are probably often idiosyncratic. Response surface experimental designs are a
368 promising approach to unravel the complexity of non-host diversity effects and the underlying
369 mechanisms, ultimately aiming to understand the relationship between community diversity and
370 disease risk.
371
372 **Fig. 1:** Differences between additive and substitutive experimental designs (above) and the
373 response surface experimental design used in this study (below). The white and grey symbols
374 indicate two different non-host species, the small blue rectangles individual experimental units. In
375 our experiments, we used a two-factorial response surface design, with two non-host species and
376 four density levels each.
377
378 **Fig. 2:** Mean number of infective cercarial stages remaining (± SD) after exposure to combinations
379 of two non-host species at different densities in response surface design experiments with A) crabs
380 and seaweed, B) seaweed and oysters, and C) oysters and crabs. Each treatment combination was
381 replicated four times, i.e. total N=64 per experiment. Note that raw data are presented here to

382 indicate the structure and variance of the data on which the analyses were based. Removal rates by
383 non-hosts and cercarial survival were calculated from the best fitting models (Tables S1-3). For
384 details, see main text.
385